PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Li *et al.* Group Art Unit: 1635

**Serial No.: 10/574,129** Examiner: Bowman, Amy Hudson

Filed: November 2, 2006 Docket No.: 180/179 PCT/US

Confirmation No.: 9249

For: A NOVEL siRNA-BASED APPROACH TO TARGET THE HIF-α FACTOR FOR GENE THERAPY

CONSIDERED: /AB/

/Amy Bowman/

10/13/2009

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DECLARATION OF CHUAN LI, PH.D. PURSUANT TO 37 C.F.R. §1.132

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. My name is Chuan Li, Ph.D., and I am Director of Molecular Radiation Oncology at the University of Colorado at Denver's School of Medicine.

2. A true and accurate copy of my *curriculum vitae*, which evidences my expertise and credentials, is attached herewith and labeled **Exhibit B**.

3. I am a co-inventor of the above captioned U.S. Patent Application Serial No. 10/574,129.

4. I have had an opportunity to review pending claims 36, 38-42, 45-59, 62, and 63 in the above captioned U.S. Patent Application Serial No. 10/574,129.

5. I have also had the opportunity to review the Non-Final Official Action dated December 18, 2008 (hereinafter the "Non-Final Official Action") from the United States Patent and Trademark Office (hereinafter "the Patent Office").

6. Various siRNAs were tested for their abilities to downregulate HIF-1α activity by producing retroviruses that encode various siRNAs that target the human HIF-1α mRNA sequence. Retroviruses encoding siRNA sequences targeted to the various sequences in the human HIF-1α gene were produced that targeted the following human HIF-1α nucleotide sequences:

ATGACATGAAAGCACAGAT (siRNA 1);

AACTGGACACAGTGTGTTT (siRNA 2);

AAATGAGAGAAATGCTTAC (siRNA 3); and

AAATGGCCTTGTGAAAAAG (siRNA 4).

7. siRNA 1 included a sense sequence that corresponded to SEQ ID NO: 7 of the above captioned U.S. Patent Application Serial No. 10/574,129. The other three siRNAs were targeted to sequences in the vicinity of the sequence targeted by siRNA 1 as shown in **Exhibit C** submitted herewith.

8. The retroviruses were used to infect the human colon cancer cell line HCT116. After infection, the cells were treated with puromycin, which selected for stable integration of the retrovirus genomes. After 5 day of selection, puromycin was washed off the cells, and the cells were subjected to hypoxia (0.5% $O_2$) treatment for 24 hours. After 24 hours treatment, the cells were lysed and assayed for HIF-1 activity using a commercially available kit purchased from Active Motf of Carlsbad, California.

9. The results of these assays are presented in **Exhibit D** submitted herewith. Normoxic cells containing each of the siRNAs were used as controls for normalization. Control indicated vector infected control cells.

10. As can be seen in **Exhibit D**, the retrovirus that encoded an siRNA that included SEQ ID NO: 7 (*i.e.*, siRNA 1) showed superior ability to downregulate HIF-1 activity as compared to any of siRNAs 2-4. Specifically, cells containing siRNAs 2, 3, and 4 showed about 50-300% higher HIF-1 activity levels than cells containing siRNA 1.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Chuan Li, Ph.D.

6/17/09

Date

Attachments: **Exhibits B-D**

# Exhibit B

*Curriculum Vitae*

## Chuan-Yuan Li

## Personal Information

***Tel:*** (303) 724-1542
***Fax:*** (303) 724-1554
***Email:*** Chuan.Li@ucdenver.edu

***Citizenship:***
U.S. Citizen.

## Education

**B.S. in Chemistry**, 1987, University of Science & Technology, Hefei, China.
**Ph.D. in Cancer Biology**, 1993, Harvard University, Cambridge, MA.

## Professional Experience

| | |
|---|---|
| 2006- | Professor of Pharmacology, University of Colorado Health Sciences Center, Aurora, CO. |
| 2006- | Professor and Director, Division of Radiation and Cancer Biology, Dept. of Radiation Oncology, University of Colorado Health Sciences Center, Denver, CO. |
| 2005-2006 | Professor, Departments of Radiation Oncology, Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC |
| 2002-2005 | Associate Professor, Dept. of Radiation Oncology, Duke University Medical Center, Durham, NC |
| 1997-2002 | Assistant Professor, Dept. of Radiation Oncology, Duke University Medical Center, Durham, NC |
| 1993-1996 | Postdoctoral research fellow, Harvard University, Boston, MA |

## Honors and Awards

| | |
|---|---|
| 1987-89 | Rohm and Haas Fellowship for graduate study at Harvard University |
| 1990 | Student Travel Award, 39th Radiation Research Society Annual Meeting, New Orleans, LA |
| 1991 | Student Travel Award, 9th International Congress of Radiation Research, Toronto, CA |
| 1993 | Kresge Center for Environmental Science pilot grant, Harvard University |
| 1999 | Komen Foundation Grant for Breast Cancer Research |
| 2001 | Best Poster Award, Annual Meeting of the Duke University Cancer Center |
| 2002 | Best Proffered Paper, 6$^{th}$ Wolfsberg Symposium in Radiation Oncology, Ematingen, Switzerland, 2002 |
| 2006 | Michael Fry Research Award from the Radiation Research Society, Philadelphia, PA. |

## Awards obtained by graduate students and postdoctoral fellows.

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|---|---|
| 2005 | AACR Travel Award, Fang Li, Postdoc Anaheim, CA |
| 2005 | RRS travel Award, Postdoc Fang Li |
| 2005 | RRS travel award, Postdoc Shanling Liu |
| 2005 | Excellence and Soundness of Research Methods award from the Fifth International Symposium on Therapeutic Ultrasound, Student: Yunbo Liu |

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| 2006 | Scholarship to attend the Keystone symposium in Hypoxia research for Fang Li, postdoc. |
| 2006 | Scholarship to attend AACR special conference on genetic susceptibility to cancer, Bin Yan. |
| 2008 | Travel Award for postdoc Wenrong Li to attend 54th annual meeting of RRS. |

## Membership in Professional Societies

Radiation Research Society, Member of the Educational Committee
American Association for Cancer Research
American Society of Gene Therapy
International Society of Stem Cell Research

## Reviewership

***Reviewers for Academic Journals:***

Molecular Cell, Cancer Research, Clinic Cancer Research, Gene Therapy, J. of National Cancer Institute, Molecular Therapy, International J. of Cancer, J. of Investigative Dermatology, Int. J. of Hyperthermia, Int. J. of Radiation Oncology, Biology and Physics, Leukemia, etc.

***Grant Review Committee Membership:***

*Ad hoc* member, NIH study section of Metabolic Pathology, 2001-2003. Radiation Therapeutics and Biology (RTB), 2003-2004, 2007-present;
Komen Foundation for Breast Cancer Research, 2000
NASA Fundamental Space Biology Research Program, 2004, 2005
DOE Low Dose Radiation Research Program, 2004
Clinical and Basic Science Grant Review Panel, National Science Foundation of China, 2005-Present.

## Invited Presentation(selected)

1. Elevated frequency of microsatellite mutations in human lymphocyte cells selected for mutation a at the thymidine kinase locus, *41st Annual Meeting of the Radiation Research Society*, Dallas, Texas, 1993.
2. Involvement of tumor suppressor genes in human cell transformation, *Workshop on Neoplatic transformation in human cell systems in culture: Mechanisms of Carcinogenesis*, Chicago, Illinois, 1995.
3. A Heat-Induced Gene Therapy Approach for Cancer Treatment, *46th Annual Meeting of the Radiation Research Society*, Dallas, Texas, 1998.
4. Hyperthermia-regulated gene therapy. Annual meeting of the North America Hyperthermia Society, Philadelphia, PA, 1999
5. Combined radiation and gene therapy treatment of breast cancer in murine tumor models, *Annual Research Meeting of the Komen Foundation for Breast Cancer Research*, Washington DC, 2000
6. Progress in hyperthermia-regulated gene therapy (Keynote Address), *The 17th Annual Meeting of the Japanese Society of Hyperthermic Oncology*, Niigata, Japan, 2000
7. Heat-controlled genet therapy, *International Conference of Stress Proteins in Biology and Medicine*, Woods Hole, MA, 2000
8. Persistent genetic instability in cancer cells induced by non-DNA damaging stress, Gordon Conference on Radiation Oncology, Ventura City, CA, 2001

9. Heat-induced transcriptional Targeting of therapeutic genes to cancer, 48th annual Meeting of the Radiation Research Society, Puerto Rico, 2001
10. Increased resistance of cancer cells mediated by the ILK gene, 49th annual meeting of the Radiation Research Society, Reno, NE, 2002
11. Tumor therapy by use of adenovirus vectors that target tumor cells genetically. 6th Wolfsberg Symposium in Radiation Oncology, Ematingen, Switzerland, 2002.
12. Telomerase-targeted oncolytic virus gene therapy. Annual Meeting of the North American Hyperthermia Society, Quebec City, Quebec, Canada. 2003.
13. Targeted gene therapy for cancer. 10th SCBA International Symposium, Beijing, China. 2004.
14. Minisyposium presentation, AACR annual meeting, Anaheim, CA ,2005.
15. Invited presentation, NIH conference on Bioimaging, DC, 2005
16. Invited presentation, Radiation Research Society, Denver, Colorado, 2005.
17. Invited Speaker, Division of Pharmaceutical Sciences, University of Wisconsin, Madison, 2006
18. Invited Speaker, Ontario Cancer Institute, Toronto, Canada, 2006

## Teaching Activities

**Cancer Biology** (1997-1999), offered by DUMC Cancer Center, Primary Instructor: Dr. EC Halperin.
**Radiation Biology**(1997-2006), offered by Dept. of Radiation Oncology, DUMC; Primary Instructor: Chuan-Yuan Li.
**Biomedical Engineering 260** (1997-2005), offered by Dept. of Biomedical Engineering, Duke University School of Engineering. Primary Instructor: Dr. F. Yuan.
Biology of Radiation Therapy, offered by Dept or Radiation Oncology, University of Colorado Denver School of Medicine. Primary Instructor: Chuan-Yuan Li.

## Grant Support

***Past Support***

Development of a novel gene therapy approach based on hyperthermia. Duke Breast SPORE pilot project. Amount: $18,000; Duration: 07/97-06/98. PI;Chuan-Yuan Li

Development of Biological Sensor for Stress exposure. Pilot project funded by the Duke University Marine Biology Center. Amount $15,000. Duration 07/98-06/99. PI: Chuan-Yuan Li

Hyperthermia-controlled gene therapy, Celsion Cooperation. Amount: $50,000; Duration 1998-1999. PI: Chuan-Yuan Li

A hyperthermia-mediated gene therapy approach for cancer. NIH 1R01CA81512. Amount: $120,000/yr direct cost. Duration: 1998-2003. PI: Chuan-Yuan Li

Isolation and Characterization of Angiogenesis-related genes in the tumor vasculature. Glaxo-Wellcome-Duke Collaborative Research Agreement. Amount: $300,000. 1999-2001. PI: Chuan-Yuan Li

Enhancement of breast cancer radiotherapy by genetic immunotherapy. Komen Foundation for Breast Cancer Research. Amount: $250,000. Duration: 1999-2001. PI; Chuan-Yuan Li

A Replication-competent Adenovirus That Targets Hypoxic Tumor Cells. Duke Comprehensive Cancer Center. Discovery Research Group Grant. Amount: $30,000. Duration: 2002. PI: Chuan-Yuan Li.

A novel transgenic mouse model for studying tumor hypoxia and angiogenesis. Morris Cancer Foundation & NIH imaging Pilot grant. Amount: $100,000. Duration: 2002-03. PI: Chuan-Yuan Li.

Hyperthermia and perfusion effects in cancer therapy. NIH 5PO1 CA42745. Amount $17,000,000. Duration: 2000-2005. PI: M.W. Dewhirst. 10% effort for C-Y. LI

SPORE in Breast Cancer. NIH 5P50-CA-68438. Amount: $1,851,120. Duration: 2002-2007. PI: Lyerly. 15% Effort for C-Y. Li

Enhancement of Prostate Cancer Radotherapy by immunogene therapy. Department of Defense. Amount: $215,000. Duration: 2002-2006. PI: Chuan-Yuan Li

Mechanistic studies of tumor therapy by electrofusion. NIH. Amount:200K/yr annual direct cost. Duration: 2002-2007. PI: F. Yuan. 10% effort for C-Y. Li.

A novel adenovirus vector that targets telomerase-expressing breast cancer cells. Komen Foundation for Breast Cancer Research. Amount: $250,000/3yrs. Duration: 2003-2007. PI; Chuan-Yuan Li

Molecular Dissection of the roles of SOD genes in mammalian cellular response to low dose radiation. DOE. Amount: $900K for 3 years. 2003-2007. PI: C-Y. Li

Ultrasound-mediated gene delivery and activation. NIH. Amount $1,250,000. Duration: 2003-2008. PI: Pei Zhong. 10% effort for C-Y. Li.

A hyperthermia-mediated gene therapy approach for cancer. NIH 1R01CA81512. Amount: $150,000/yr direct cost. Duration: 2003-2009. PI: Chuan-Yuan Li

HZE particle induced persistent genetic instability/oncogenic transformation and their prevention. National Aeronautics and Space Administration (NASA). Amount: 200K/yrs. Duration: 2003-2008. PI: C-Y. Li

Imaging tumor hypoxia in a transgenic mouse model. NIH R21 EB001882. Amount: $500,000 total. Duration: 2003-2007 . PI: Chuan-Yuan Li

***Active/Current Support***

University of Colorado SPORE in Lung Cancer (NCI). PI: Paul Bunn. Amount: $2,500,000/yr. Duration: 2008-2013. Part for C-Y. Li, Developmental Project: $50,000/yr direct cost.

Roles of HIF-1 genes in head and neck cancer radiotherapy (NIH/NCI). PI: Chuan-Yuan Li, Duration: 01/01/2009-12/31/2013. Amount: $200K/yr direct, $300K/yr total.

Mechanistic studies of space radiation induced carcinogenesis (NASA). PI: Chuan-Yuan Li, Duration: 06/01/2009-05/31/2010. Amount: $220K/yr direct, $354K/year total.

Mechasnistic studies of tumor response to cytotoic chemotherapy (NIH/NCI). Duration: 07/01/2009-06/30/2014; PI: Chuan-Yuan Li; Amount: 200K/yr direct, $300K/yr total.

## Publications

***Peer-Reviewed Publications:***

1. **Li, C-Y.**, Yandell, D.W., and Little, J.B. Molecular Mechanisms of Spontaneous and Induced Loss of Heterozygosity in Human Cells In Vitro (1992). *Somatic Cell and Molecular Genetics,* Vol. 18, 77-87.

2. **Li, C-Y.**, Yandell, D.W., and Little, J.B. Evidence for Coincident Mutations in Human Lymphoblast Clones Selected for Functional Loss of a Thymidine Kinase Gene(1992). *Molecular Carcinogenesis,* Vol. 5, 270-277.

3. Spiro, I. J., Yandell, D.W., **Li, C-Y.** , Saini, S., Ferry, J., Powelson J., Katkov, W.N., and Cosimi, A.B. Lymphoma of Donor Origin Occuring at the Porta Hepatis of Transplanted Liver(1993). *New England Journal of Medicine,* Vol.329, 27-29.

4. Poremba, C., Dockhorn-Dworniczak, B., Merrit, V., **Li, C-Y.**, Heidle, G., Tauber, P., Bocker, W., , and Yandell, D.W. Immature Teratomas of Different Origin Carried by a Pregnant Mother and Her Fetus(1993). *Diagnostic Molecular Pathology* , Vol.2, 131-136.

5. **Li, C-Y.**, Yandell, D.W., and Little, J.B. Elevated frequency of Microsatellite Mutations in Human Lymphoblast Clones Selected for Mutations at Thymidine Kinase locus(1994). *Molecular & Cellular Biology* , Vol. 14, 4373-4379.

6. **Li, C-Y.**, Nagasawa, H., and Little, J.B. Confluent Holding Recovery in Irradiated Human Diploid Fibroblasts: Possible Role of the p53/Waf1 Signal Transduction Pathway(1995). *Journal of Cellular Biochemistry*, (S) 21A, 340.

7. **Li, C-Y.**, Nagasawa, H., Tsang, N., and Little, J.B. Radiation-Induced Irreversible G0/G1 Block is Abolished in Human Diploid Fibroblasts Transfected with the Human Papilloma Virus E6 gene: Implication of the p53-Cip1/WAF1 pathway (1995). *International Journal of Oncology*, Vol. 223-236.

8. **Li, C-Y.**, Suardet, L., and Little, J.B. Potential Role of WAF1/Cip1/p21 as a Mediator of TGF-b Cytoinhibitory Effect . *Journal of Biological Chemistry*, Vol. 270 No. 10., 1995 pp4971-4974.

9. Nagasawa, H., **Li, C-Y.**, Maki, C.G. , Imrich A. and Little, J.B. Relationship between Radiation-Induced G1 Phase Arrest and p53 Function in Human Tumor Cells *Cancer Research* vol 55 No. 9, 1995, pp1842-1846.

10. Little, J.B. , Nagasawa, H., Keng, P., and **Li., C-Y.** Absence of Radiation-induced G1-arrest in Two Closely Related Human Lymphoblast Cell Lines That Differ in p53 Status *Journal of Biological Chemistry* Vol.270, No. 19, 1995 pp11033-11036.

**11.** Tsang, N., Nagasawa, H., **Li, C-Y.**, and Little, J.B. Abrogation of p53 function by Transfection of HPV16 E6 Gene Enhances the Resistance of Human Diploid Fibroblasts to Ionizing Radiation *Oncogene* vol. 10, 1995, pp2403-2408.

**12. Li, C-Y.**, Nagasawa, H., Dahlberg, W.A., and Little, J.B. Diminished Capacity for p53 in Mediating a Radiation-Induced $G_1$ Arrest in Established Human Tumor Cell Lines *Oncogene* vol. 9, No. 9, 1995, pp1885-1892.

13. Little, J.B., **Li., C-Y.**, Nagasawa, H., Pfenning, T., and Vetrovs, H. Genomic Instability and Radiation Mutagenesis(1996) *J. Chimie Phys.* vol. 93, 157-164.

14. **Li, C-Y**, Nagasawa, H., Dahlberg, W.A., and Little, J.B. The Role of Tumor Suppressor Genes in Determining a Radation-induced $G_1$-arrest and Human Cell Carcinogenesis(1996) *Radiation Oncology Investigations*, vol. 3, 268-271.

15. Huang, H-M., **Li, C-Y**, Little, J.B. Abrogation of p53 Function in Human Tumor Cells Does Not Alter Their Sensitivity to Ionizing Radiation(1996). *International Journal of Radiation Biology,* vol. 70 (2), 151-160.

16. Suardet, L., **Li, C-Y.,** and Little J.B. Radio-induced modulation of transforming growth factor sensitivity in a p53 wild-type human colorectal cancer cell line(1996) . *International Journal of Cancer* vol. 68(1), 126-31.

**17.** Huang, Q., Tao., Y., **Li, C-Y.,** and Yandell, D. The status of p16 and p15 in primary tumors and cell lines (1996). *Chinese Journal of Medical Genetics*, vol. 13, 198-202.

**18.** Yu, Y., **Li, C-Y.**, Little, J.B. Abrogation of p53 function by HPV16 E6 gene delays apoptosis and enhances mutagenesis but does not alter radiosensitivity in TK6 human lymphoblastoid cells(1997). *Oncogene*, vol. 14(14), 1661-1667.

19. Little JB, **Li C-Y**, Nagasawa H, Huang H. Influence of p53 expression on radiosensitivity of human normal and tumor cells(1998). *J CHIM PHYS* vol 95,820-829.

20. Tao, Y., Huang, Q., **Li, C-Y.**, and Yandell, D. Deletions and point mutations of p16, p15 gene in primary tumors and tumor cell lines. (1999). Chinese Med Sci. J. vol.14, 200-235.

21. Huang, Q., Shan, S, Braun, R.D., Lanzen, J., Anyrhambatla, G., Kong, G., Borelli, M., Corry, P., Dewhirst, M.W., and **Li, C-Y.** GFP based non-invasive, in vivo monitoring of gene expression(1999). *Nature Biotechnology*, vol 17, 1033-1035.

**22. Li, C-Y.,** Huang, Q., Braun, R.D., Lanzen, J., Hu, K., Lin, P., Dewhirst, M.W. Initial stages of tumor cells-induced angiogenesis:evaluation via skin window chambers in rodent models(2000) *Journal of the National Cancer Institute*, vol 92, 143-127.

23. Huang, Q., **Li, C-Y.** Comparative studies of GFP gene transgene by viral and non-viral approaches (2002). *Chinese J. Pathology.* Vol 29, 143-144.

24. Huang, Q., **Li, C-Y.** Dynamic observation of tumor cell growth by use of GFP-transduced tumor cells (2000). *Chinese J. Pathology.* Vol 29, 230-232.

25. Huang, Q., Xu, P., Liu, WW., Wang, F., Gu, Q., Tian, SH., Fan, Y., Xie, KC., Chen, XF., Li, C-Y. Preparation of green fluorescent protein retrovirus and its application in mediating gene transfer into retinal pigment epithelial cells (2001). *Chinese J. of Ophthalmology*, vol. 37, 248-251.

26. **Li, C-Y.,** Huang, Q., Braun, R.D., Lanzen, J., Hu, K., Lin, P., Dewhirst, M.W. Re: Initial stages of tumor cell-induced angiogenesis: Evaluation via skin window chambers in rodent models - Response(2000). *Journal of the National Cancer Institute,* Vol. 92, 1445-1446.

27. Huang,Q., Hu, K., Lohr, F., Zhang,L., Braun, R., Lanzen,J., Little,JB., Dewhirst,MW., and **Li, C-Y**Heat-induced Gene Expression as a Novel Targeted Cancer Gene Therapy Strategy.(2000) *Cancer Research,* vol. 60, 3435-3439.

*28.* Lohr, F., Hu, K., Huang, Q., Zhang, Li., Dewhirst, MW., and **Li, C-Y.** Enhancement of Radiotherapy by Hyperthermia-regulated Gene Therapy.(2000). *International Journal of Radiation Oncology, Biology and Physics.* Vol.48, 1513-1518.

*29.* Lohr,F., Hu, K., Haroon,Z., Samulski, TV., Huang,Q., Beaty,J., Dewhirst,MW., and **Li, C-Y.** Combination treatment of murine tumors by adenovirus mediated local B7/IL12 immunotherapy and radiotherapy(2000). *Molecular Therapy*, vol2, 195-203.

*30.* **Li, C-Y.,** Shan, S., Cao, Y., Dewhirst, M.W. Role of incipient angiogenesis in cancer metastasis.(2000). *Cancer Metastasis Reviews*, vol 19, 7-11.

*31.* **Li, C-Y.,** Little, JB. Hu, K., Zhang, W., Zhang, L., Dewhirst, MW., Huang, Q. Persistent genetic instability in cancer cels induced by non-DNA damaging stress exposures (2001). *Cancer Research* vol 60, 428-432

*32.* Zhang, X., and **Li, C-Y.** Generation of recombinant adeno-associated virus vectors by a complete adenovirus-mediated approach**(2001)**. *Molecular Therapy* vol.3, 787-792.

*33.* Xie, KC., Xu, P., Gu, Q., Liu, WW., Wang, F., Tian SH., Chen, XF., **Li, C-Y.,** Huang, Q. Using GFP retrovirus to label tumor cells and vascular endothelial cells. *Chinese J. Lung Cancer.* Vol 4, 20-24.

34. Lohr,F., Lo, D., Zaharoff, DA., Hu, K., Li, Y., Zhang, X., Dewhirst, MW., Yuan, F., and **Li, C-Y.** Effective tumor therapy with plasmid-encoded IL12 and IL2 combined with in vivo electroporation **(2001)** *Cancer Research vol* 61, 3282-3285

35. Huang, Q., and **Li, C-Y.** Using hsp70 promoter to regulate target gene expression in tumor. *Chinese J. of Pathology.*, vol. 30, 198-201.

36. Lohr, F., Huang, Q., Hu, K., Dewhirst, MW., and **Li, C-Y.** An inducible method for preventing systematic effects of toxic therapeutic genes delivered by intratumorally injected adenovirus vectors. *Clinical Cancer Research.* Vol. 11, 3625-3628

37. Zhang, X., Hu, K., and **Li. C-Y.** Protection against oxidized LDL-induced vascular endothelial cell death by integrin-linked kinase (**2001**). *Circulation* vol 104, 2762-2766.

*38.* Zaharoff, D., Barr, R., **Li, C-Y.**, Yuan, F. Mechanistic studies of electric pulse mediated gene transfer in murine tumors (2002). *Gene Therapy.* Vol. 9, 1286-1290.

39. Wang, F., Chen, X., Tian, Y., Wu, J., Li, L., **Li, C-Y.**, Huang, Q. Target gene transfer mediated by electroporation for cancer therapy in vivo (2002). *Progress in Biochemistry and Biophysics.* Vol. 29, 734-740.

40. **Li, C-Y.,** and Dewhirst, M.W. Hyperthermia-regulated immunogene therapy (2002). *International Journal of Hyperthermia* Vol. 18, 589-96.

41. **D**ewhirst, M.W., Cao, Y., Moeller, B., **Li, C-Y.** Intravital Fluorescence facilitates the measurement of multiple physiological parameters and gene expression in tumors of living animals. *Disease Markers.* 18 (5-6): 293-311 2002

**42.** Azzam, EI, Nagasawa,N., Yu,Y., **Li, C-Y.** and Little, JB. Cell Cycle Deregulation and XPC Cell Transformation (2002). *Journla of Investigative Dermatology.* Vol 119, 1350-1354.

**43.** Baker-LePain, J.C., Sarzotti-Kelsoe, M., Fields, T.A., **Li, C-Y.**, Nicchita, C.V. GRP94(gp96) and GRP94 N-Terminal binding domain elicit tissue non-restricted tumor suppression.(2002) *Journal of Experimental Medicine* Vol. 196, 1447-59.

**44.** Wang, F., Tian, Y., Li, L., Chen, X., Hu, HH. **Li, C-Y.**, Huang, Q. Inhibition of tumor angiogenesis, growth, and metastasis by blocking VEGF paracrine pathway (2002). *Acta Biochimica et Biophysica Sinica,* vol 34.,165-170.

**45.** Zhang, X., Li, Y., Yan, B., Huang, Q., Dewhirst, M.W., **Li, C-Y.** Increased resistance of tumor cells to hyperthermia mediated by integrin-linked kinase (2003). *Clinical Cancer Research* Vol 9, 1155-60.

**46.** Wang, Y., Hu, JK., Krol, A., Li, Y-P., **Li, C-Y.**, Yuan, F. Systemic dissemination of viral vectors during intrtumoral injection.(2003). *Molecular Cancer Therapeutics.* Vol. 1233-1241.

47. Wang, F., Wu, J., Tian, Y.,Chen, X., Hu, H., Wu, W., **Li, C-Y.**, and Huang, Q. Role of VEGF in the growth and metastasis of a murine bladder carcinoma. (2003) *Chinese Science Bulletin.* Vol 48:2404-2410.

48. Huang, Q., Wang, H., Zhang, XW., Yan, B., Dewhirst, MW., and **Li, C-Y.** A conditionally replicative adenovirus targeted to telomerase-positive cancer cells. (2004)*Clinical Cancer Research.* Vol: 4:1439-1445

49. Shan, S., Robson, N. D., Cao, Y., Qiao, T., **Li, C. Y.,** Kontos, C. D., Garcia-Blanco, M., Dewhirst, M. W. Responses of vascular endothelial cells to angiogenic signaling are important for tumor cell survival.(2004) *Faseb Journal.* Vol : 17(15) published online.

*50.* Zhang, X., Huang, Q., Li, Y., Yang, H., and **Li, C-Y.** GW112, a novel gene involved in the survival of cancer cells under stressful conditions. (2004)*Cancer Research.* Vol. 64: 2474-2481.

*51.* Moeller, B. J.; Cao, Y., **Li, C. Y.,** Dewhirst, M. W. Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors; Role of reoxygenation, free radicals, and stress granules (2004) *Cancer Cell.* Vol 5: 429-441.

*52.* Moeller, B.J., Cao, Y., Vujaskovic, Z., **Li, C.Y.,** Haroon, Z.A., Dewhirst, M. W.(2004) The relationship between hypoxia and angiogenesis. *Seminars in Radiation Oncology.* Vol 14:215-21.

*53.* Herskind C, Fleckenstein K, Lohr J, **Li CY**, Wenz F, Lohr F (2004) Antitumoral action of interferons and interleukins in combination with radiotherapy. Part 1: Immunologic basis. *STRAHLENTHERAPIE UND ONKOLOGIE 180 (4): 187-193*

*54.* Herskind C, Fleckenstein K, Lohr J, **Li CY**, Frederik W, Lohr F. (2004) Antitumoral action of Interferons and interleukins in combination with radiotherapy. part II: Radiobiological and immunologic strategies. *STRAHLENTHERAPIE UND ONKOLOGIE* 180 (6): 331-339

**55.** Zhang,X., Wang, H., Kon, T., Huang, Q., Dewhirst, MW., and **Li, C-Y.** Enhancement of tumor cell death in vitro and radiation therapy in vivo by use of an siRNA targeted to HIF1-α (2004). *Cancer Research.* Vol 64:8139-42.

**56.** V. Rao, P. Deng, R. Maddala, D. Epstein, **C-Y. Li,** Hiroaki Shimokawa (2005) Expression of dominant negative Rho-binding domain of Rho-kinase in organ cultured human eye anterior segments increases aqueous humor outflow. *Molecular Vision,* Vol 11: 288-97.

**57.** Wang, Y., Yang, Z., Liu, S., Krol, A., **Li, C-Y.**, and Yuan, F (2005). Characterization of systemic adenovirus leakage during cancer gene therapy. *British Journal of Cancer.* Vol 92:1414-20.

**58. Li, C. Y.,** Huang, Q., Kung, H. F. (2005) Cytokine and immuno-gene therapy for solid tumors. *Cell & Molecular Immunology.* Vol 2: 81-91.

**59.** Cao, Y., **Li, C-Y.**, Moeller, B.J., Yu, D., Zhao' Y, Dreher,M., Shan., S., and Mark W. Dewhirst(2005). Observation of incipient angiogenesis that is independent of hypoxia and HIF-1 activation *Cancer Research. Vol.* 65: 5498-5505

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# Exhibit C

**SEQ ID NO: 1 – Human HIF-1α Coding Sequence**

```
atg gag ggc gcc ggc ggc gcg aac gac aag aaa aag ata agt tct gaa
Met Glu Gly Ala Gly Gly Ala Asn Asp Lys Lys Lys Ile Ser Ser Glu
                5                  10                  15

cgt cga aaa gaa aag tct cga gat gca gcc aga tct cgg cga agt aaa
Arg Arg Lys Glu Lys Ser Arg Asp Ala Ala Arg Ser Arg Arg Ser Lys
            20                  25                  30

gaa tct gaa gtt ttt tat gag ctt gct cat cag ttg cca ctt cca cat
Glu Ser Glu Val Phe Tyr Glu Leu Ala His Gln Leu Pro Leu Pro His
        35                  40                  45

aat gtg agt tcg cat ctt gat aag gcc tct gtg atg agg ctt acc atc
Asn Val Ser Ser His Leu Asp Lys Ala Ser Val Met Arg Leu Thr Ile
    50                  55                  60

agc tat ttg cgt gtg agg aaa ctt ctg gat gct ggt gat ttg gat att
Ser Tyr Leu Arg Val Arg Lys Leu Leu Asp Ala Gly Asp Leu Asp Ile
65                  70                  75                  80
```

**SEQ ID NO: 7 (siRNA 1)**

```
gaa gATGACATGAAAGCACAGATg aat tgc ttt tat ttg aaa gcc ttg
Glu Asp Asp Met Lys Ala Gln Met Asn Cys Phe Tyr Leu Lys Ala Leu
                85                  90                  95

gat ggt ttt gtt atg gtt ctc aca gat gat ggt gac atg att tac att
Asp Gly Phe Val Met Val Leu Thr Asp Asp Gly Asp Met Ile Tyr Ile
            100                 105                 110

tct gat aat gtg aac aaa tac atg gga tta act cag ttt gaa ctaact
Ser Asp Asn Val Asn Lys Tyr Met Gly Leu Thr Gln Phe Glu Leu Thr
        115                 120                 125
```

**siRNA 2**

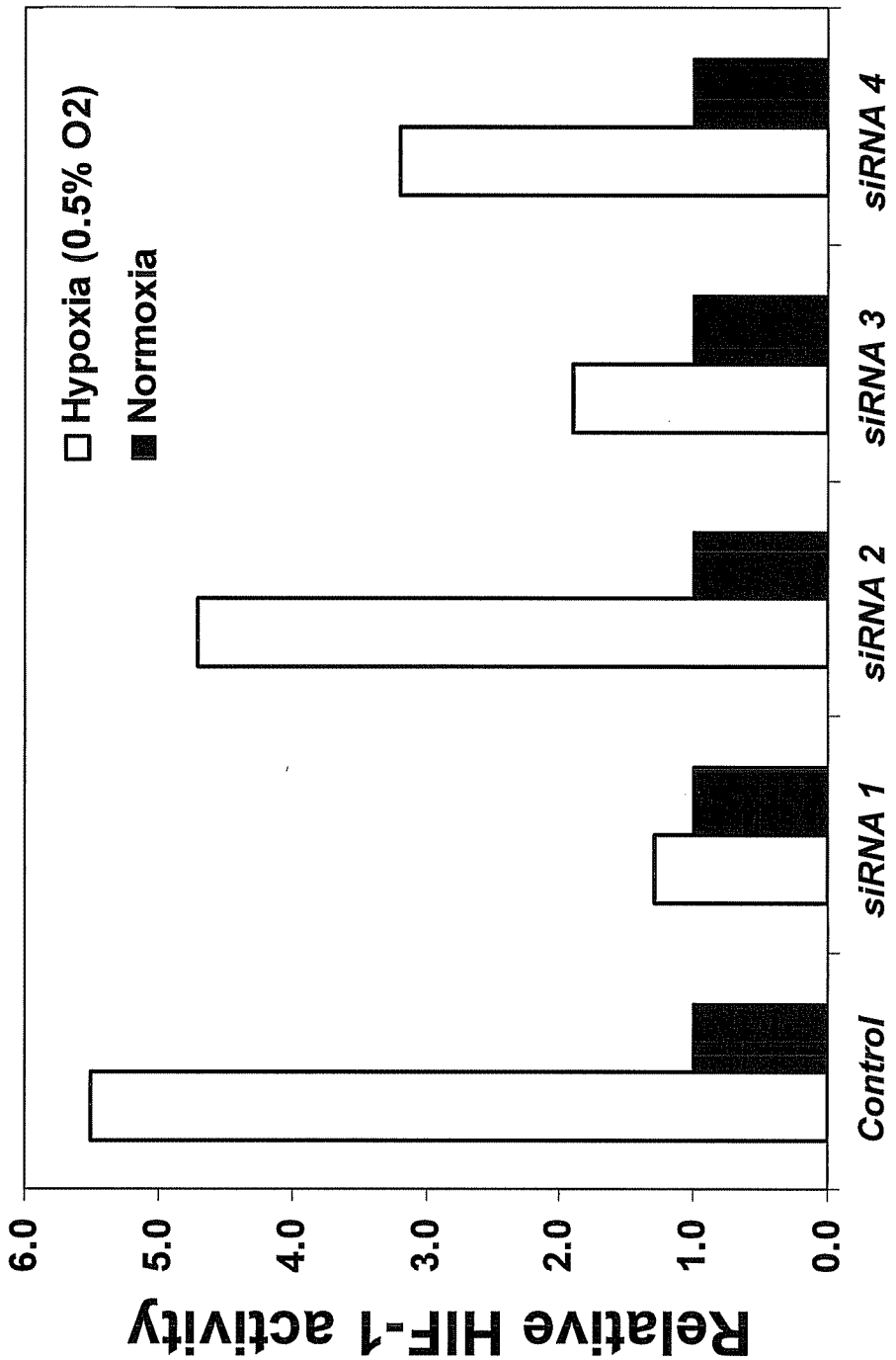
```
ggacacagtgtgttt gat ttt act cat cca tgt gac cat gag gaaatg
Gly His Ser Val Phe Asp Phe Thr His Pro Cys Asp His Glu Glu Met
    130                 135                 140
```

**siRNA 3** **siRNA 4**

```
agagaatgcttaca cac agaatggcttgtgaaaag ggt aaa gaa
Arg Glu Met Leu Thr His Arg Asn Gly Leu Val Lys Lys Gly Lys Glu
145                 150                 155                 160

caa aac aca cag cga agc ttt ttt ctc aga atg aag tgt acc cta act
Gln Asn Thr Gln Arg Ser Phe Phe Leu Arg Met Lys Cys Thr Leu Thr
                165                 170                 175
```

**Exhibit D**


Relative HIF-1 activity
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Hypoxia (0.5% O2)
Normoxia
Control
siRNA 1
siRNA 2
siRNA 3
siRNA 4